OIPE JC17
APR 17 2002
PATENT & TRADEMARK OFFICE

OF PAPERS
GINALLY FILED

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TREATMENT OF CENTRAL NERVOUS SYSTEM DISEASES BY ANTIBODIES AGAINST GLATIRAMER ACETATE

RECEIVED
APR 2 4 2002
OFFICE OF PETITIONS

the specification of which:
(check one)

___ *is attached hereto.*

_X_ *was filed on* June 20, 2001 *as*

Application Serial No. 09/885,227

and was amended _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s)			*Priority Claimed*	
Number	*Country*	*Filing Date*	*Yes*	*No*
N/A			___	___
			___	___
			___	___
			___	___
			___	___
			___	___

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

Provisional Application No.	Filing Date	Status
60/287,171	April 27, 2001	Pending
60/269,788	February 16, 2001	Abandoned
60/212,577	June 20, 2000	Abandoned

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
N/A		

And I hereby appoint

John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); Jay H. Maioli (Reg. No. 27,213); William E. Pelton (Reg. No. 25,702); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Wendy E. Miller (Reg. No. 35,615); Richard S. Milner (Reg. No. 33,970); Robert T. Maldonado (Reg. No.38,232); Paul Teng (Reg. No. 40,837); Richard F. Jaworski (Reg. No. 33,515); Alan J. Morrison (Reg. No. 37,399); Mark A. Farley (Reg. No. 33,170); Pedro C. Fernandez (Reg. No. 41,741); Gary J. Gershik (Reg. No. 39,992); Alan D. Miller (Reg. No. 42,889); and Frank Bruno (Reg. No. 46,583)

and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications and direct all telephone calls regarding this application to:

John P. White Reg. No. 28,678

Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor Moses Rodriguez (Yehudah Livneh, on behalf of and as agent for)

Inventor's signature [signature]

Citizenship United States Date of signature April 2, 2002

Residence 2402 Hillside Lane, S.W., Rochester, MN 55902

Post Office Address same

Signed by Yehudah Livneh, an authorized official of Teva Pharmaceutical Industries, Ltd., the owner by written agreement to assign or otherwise having sufficient proprietary interest in the subject application justifying this action, on behalf of and as agent for Moses Rodriguez

Full name of joint inventor (if any) Moses Rodriguez (Uzi Karniel, on behalf of and as agent for)

Inventor's signature [signature]

Citizenship United States Date of signature April 2, 2002

Residence 2402 Hillside Lane, S.W., Rochester, MN 55902

Post Office Address same

Signed by Uzi Karniel, an authorized official of Teva Pharmaceutical Industries, Ltd., the owner by written agreement to assign or otherwise having sufficient proprietary interest in the subject application justifying this action, on behalf of and as agent for Moses Rodriguez.

Full name of joint inventor (if any) Daren Ure (Yehudah Livneh, on behalf of and as agent for)

Inventor's signature [signature]

Citizenship Canadian Date of signature April 2, 2002

Residence 2315 22nd Street NW, Rochester, Minnesota, USA 55901

Post Office Address same

Signed by Yehudah Livneh, an authorized official of Teva Pharmaceutical Industries, Ltd., the owner by written agreement to assign or otherwise having sufficient proprietary interest in the subject application justifying this action, on behalf of and as agent for Daren Ure.

Full name of joint inventor (if any) Daren Ure (Uzi Karniel, on behalf of and as agent for)

Inventor's signature

Citizenship Canadian Date of signature April 7, 2002

Residence 2315 22nd Street NW, Rochester, Minnesota, USA 55901

Post Office Address same

Signed by Uzi Karniel, an authorized official of Teva Pharmaceutical Industries, Ltd., the owner by written agreement to assign or otherwise having sufficient proprietary interest in the subject application justifying this action, on behalf of and as agent for Daren Ure.

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TREATMENT OF CENTRAL NERVOUS SYSTEM DISEASES BY ANTIBODIES AGAINST GLATIRAMER ACETATE

the specification of which:
(check one)

___ *is attached hereto.*

X *was filed on* June 20, 2001 *as*

Application Serial No. 09/885,227

and was amended _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s)			*Priority Claimed*	
Number	*Country*	*Filing Date*	*Yes*	*No*
N/A			___	___
			___	___
			___	___
			___	___
			___	___
			___	___

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

Provisional Application No.	Filing Date	Status
60/287,171	April 27, 2001	Pending
60/269,788	February 16, 2001	Pending
60/212,577	June 20, 2000	Pending

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which become available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
N/A		

And I hereby appoint

John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); Jay H. Maioli (Reg. No. 27,213); William E. Pelton (Reg. No. 25,702); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Wendy E. Miller (Reg. No. 35,615); Richard S. Milner (Reg. No. 33,970); Robert T. Maldonado (Reg. No.38,232); Paul Teng (Reg. No. 40,837); Richard F. Jaworski (Reg. No. 33,515); Alan J. Morrison (Reg. No. 37,399); Mark A. Farley (Reg. No. 33,170); Pedro C. Fernandez (Reg. No. 41,741); Gary J. Gershik (Reg. No. 39,992); Alan D. Miller (Reg. No. 42,889); and Frank Bruno (Reg. No. 46,583)

and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

John P. White Reg. No. 28,678

Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor Moses Rodriguez

Inventor's signature _____

Citizenship United States Date of signature _____

Residence 2402 Hillside Lane, S.W., Rochester, MN 55902

Post Office Address same

Full name of joint inventor (if any) Daren Ure

Inventor's signature _____

Citizenship Canadian Date of signature _____

Residence 2315 22nd Street NW, Rochester, Minnesota, USA 55901

Post Office Address same

MAYO

EXECUTED

CONTRACT

PARTIES:	1 TEVA PHARMACEUTICAL (PTY) LTD 2 MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
TYPE:	AGREEMENT
PRODUCT:	COPAXONE®
TERRITORY:	NOT APPLICABLE
DATE:	1 SEPTEMBER 1997
TERM:	PROJECT SHALL BE COMPLETED BY NOT LATER THAN 24 MONTHS FROM COMMENCEMENT OF THIS AGREEMENT
NOTICE	WRITTEN NOTICE
TEVA CONTACT:	BEN ZION WEINER

AGREEMENT

between

TEVA PHARMACEUTICAL INDUSTRIES LTD.
a limited liability company incorporated under the laws of Israel, of 5 Basel Street, Petah Tiqva 49131, Israel

("Teva")

and

MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH
200 First Street SW, Rochester, MN 55905, USA
("the Institute")

1. Teva hereby appoints the Institute to perform a collaborative study ("the Project") as set out in Appendix A, which forms an integral part hereof. The Project shall be under the direction and supervision of Dr. Moses Rodriguez, principal investigator

2. The Project shall be completed by the Institute by not later than 24 months from commencement of this Agreement, unless otherwise agreed between the parties. Teva shall have the right to terminate the Agreement for any reason by a written notice.

3. As consideration for the undertaking by the Institute of the Project, Teva shall pay to the Institute the amount set out in Appendix B. Payment shall be effected in installments as set out in Appendix B.

4. All documentation, information and samples conveyed by Teva to the Institute including without limitation know-how and other data related to Copaxone® ("the Confidential Information") in relation to the Project, or otherwise, shall be treated by the Institute as strictly confidential and shall not be disclosed to any person or body. Excluded from the scope of confidential information shall be information which:

   4.1 can be shown by the recipient of such information that it was in the recipient's possession prior to disclosure by the other party in terms hereof; or

   4.2 is within or otherwise enters the public domain.

5. The Institute reserves the right to publish the results of the Project, subject to the protection and non-disclosure of the Confidential Information. The Institute will submit the manuscript of any proposed publication to Teva at least thirty (30) days before submission for publication, and Teva shall have the right ~~as a precondition to any proposed publication by the Institute~~ to review and

comment upon the publication in order to protect the Confidential Information. ~~All comments by Teva will be implemented by the Institute and all confidential proprietary~~ Information of Teva shall, upon Teva's request, be deleted from the publication prior to its publication. Upon Teva's request, publication will be delayed up to sixty (60) additional days to enable Teva to secure adequate intellectual property protection of any aspect or part of the proposed publication prior to its publication. *Institute will give reasonable consideration to all comments made by Teva regarding the publication.

6. On completion of the Project the Institute shall promptly provide Teva with a written report of the results of the Project. Teva shall be entitled, within reason, to request interim written reports from the Institute.

7. It is recorded, for the avoidance of doubt, that the Project is for research purposes only, and that it shall not be construed as a clinical study in humans of whatsoever nature.

8. Except as otherwise expressly provided by clause 5 above, the Institute hereby grants Teva the sole and exclusive right to use all of the results of the Project on a worldwide basis, in such manner as Teva, in its sole discretion, deems fit.

9. In the conduct of the Project, the Institute shall be an independent contractor and shall not be authorized or empowered to act as agent for Teva. Neither party shall enter into any contract, warranty or representation as to any matter on behalf of the other party and neither party shall be bound by the acts or conduct of the other party.

10. The Institute shall have sole responsibility at its cost for any and all claims, costs or liabilities for any loss, damage, injury or loss of life attributable to the negligence or willful misconduct of the Institute.

11. Teva shall indemnify, defend, and hold harmless the Institute, its trustees, officers, employees, and agents from (i) any claims, loss or damage arising from Teva's use of the research performed under this Agreement, and (ii) any liability and expenses arising out of any injury or condition allegedly caused by the administration of the drug or device being tested. Notwithstanding the above, Teva shall not be responsible for indemnifying the Institute, its trustees, officers, employees, or agents for any liability proven to be due to the Institute's negligence, willful misconduct, or research contrary to the protocol. In the event that Teva defends the Institute, and proof of the foregoing is established, the Institute shall reimburse Teva for all costs and expenses incurred by Teva in such defence or in payment of any claim of a third party. Teva agrees not to compromise or settle any claim against the Institute without the prior written approval of the Institute.

12. This Agreement shall be construed in accordance with the laws of New York and the federal courts of the southern district of New York shall have exclusive jurisdiction with regard to all matters arising out of this Agreement.

13. This Agreement shall not be assigned by either party without the prior written consent of the other party.

14. Any Agreement changing the terms of this Agreement in any way shall be valid only if the changes are made in writing and signed by both parties.

15. This Agreement, including its appendices, represents the entire agreement between the parties.

16. All notices and communications shall be addressed to the respective parties as follows:

If to Teva: TEVA PHARMACEUTICAL INDUSTRIES LTD.
P.O.Box 8077, Kiryat Nordau, Netanya, Israel
Dr. Rivka Riven-Kreitman
Tel: 972-9-639794 . Fax: 972-9-653778

If to the Institute: Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester MN 55905, USA
Dr. Moses Rodriguez
Tel: 001-507-284-5365 Fax: 001-507-284-4795

TEVA
PHARMACEUTICAL INDUSTRIES LTD.

Name: Ben Zion Weiner, Ph.D.
Title: Corporate Vice President Research & Development
Signature:

Name:
Title:
Signature:
Date: 14.8.96

MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH

Name: Ames W. Putnam
Title: Assistant Treasurer
Signature:

Reviewed / Contract No.

Name:
Title:
Signature:
Date:

AGREED AND CONFIRMED

Principal Investigator: Dr. Moses Rodriguez
Dated: 11/9/96

APPENDIX A

THE STUDY

Grant Proposal to be submitted to: Teva Pharmaceutical Industries - Israel

Title: Copolymer I (Cop-I) and the Promotion of Central Nervous System Remyelination

Investigator: Moses Rodriguez, M.D., Professor of Neurology, Mayo Clinic, Rochester, MN, USA 55905

E-Mail: rodriguez@mayo.edu

Fax No.: 507-284-1637

Telephone No.: 507-284-4663

BACKGROUND

A synthetic polypeptide (Cop-I) composed of alanine, glutamic acid, lysine, and tyrosine has been demonstrated recently in placebo-controlled trials to alter the natural history of multiple sclerosis (MS) (1). Nearly three decades ago investigators at the Weismann Institute of Science in Israel demonstrated this compound to be non-encephalogenic and non-toxic in laboratory animals, but yet could suppress experimental allergic encephalomyelitis (2,3). This compound has achieved robust statistical efficacy in MS using stringent intent to treat analysis for the primary endpoint of reducing clinical exacerbations. A 25% reduction in the rate of relapses was observed in the treated group compared to the placebo group. The mechanisms by which Cop-I works to suppress attacks of MS is unknown. Theoretically Cop-I may disrupt the MHC/TCR complex formation to specific peptides (4) or the drug may induce MBP- specific suppressor cells in vivo (5). Cop-I may also bind directly to major histocompatibility complex class II to replace MBP peptides (6,7).

One alternative hypothesis of how Cop-I may be effective in MS is by actively inducing a protective humoral immune response. Preliminary data obtained from Teva Pharmaceuticals (Rivka Riven-Kreitman) indicates that patients treated with Cop-I develop very high antibody titers to Cop-I. There appears to be a strong positive correlation between the presence of antibodies against Cop-I and therapeutic efficacy. This result is paradoxical when considering the hypothesis that Cop-I may interfere with antigen processing and presentation to suppress autoimmunity. This raises the possibility that Cop-I antibodies may immunomodulate the disease.

Our laboratory has been interested in developing novel strategies to promote central nervous system remyelination (8). We have used the Theiler's murine encephalomyelitis virus model of demyelination (a picornavirus) which causes a chronic progressive immune-mediated CNS demyelinating disease similar pathologically and clinically to MS (9). Using this animal model we showed that the passive transfer of CNS specific anti-

serum (10) and purified immunoglobulins (11,12) directed against myelin components promoted CNS remyelination. This contrasts to the conventional view of the humoral immune response playing a pathogenic role in CNS demyelination (13). We have also generated a monoclonal antibody that reacts against a surface component of oligodendrocytes which promotes remyelination (14-16). In particular relevance to this proposal, we demonstrated that immunoglobulins reactive with myelin basic protein also promote CNS remyelination (17). In these experiments infected SJL mice were treated with the whole anti-serum or affinity purified mouse immunoglobulins directed against rabbit or rat myelin basic proteins. There was extensive evidence for new myelin synthesis as measured by quantitative morphometry. Electron microscopy revealed numerous oligodendrocytes and remyelinated CNS axons with a relative lack of inflammatory cells. Of interest, viral antigen persisted in these animals despite enhanced CNS remyelination. These findings indicated for the first time that immunoglobulins reactive against a myelin autoantigen and in particular, myelin basic proteins, have the potential for myelin repair.

Based on our observations on the role of immunoglobulins is promoting remyelination, we considered the possibility that one potential mechanism of how Cop-I may be efficaciously in MS is by enhancing CNS repair. We propose that treatment with Cop-I induces the production of polyreactive polyclonal antibodies directed against Cop-I. These immunoglobulins could then serve as immunomodulatory or stimulatory molecules to induce CNS remyelination. The purpose of these experiments is to test this hypothesis directly using the Theiler's virus model of demyelination

SPECIFIC AIMS

1. To determine if treatment of TMEV-infected SJL mice with Cop-I: (a) prevents the development of CNS demyelination, (b) reduces the extent of demyelination once the disease is established, or (c) promotes CNS remyelination.
2. To determine whether polyclonal or monoclonal antibodies directed against Cop-I promote CNS remyelination.

EXPERIMENTAL DESIGN

Experiment 1: **Does Cop-I inhibit CNS demyelination induced by Theiler's virus?** Before addressing whether Cop-I enhances remyelination, it is important to test whether or not Cop-I will affect the demyelination induced by Theiler's virus. In this experiment Cop-I treatment will be begun prior to infection with Theiler's virus and will continue throughout the course of the infection. Mice will be treated subcutaneously daily with 1 mg of Cop-I. This dosage simulates the clinical situation in patients. We will also treat a second group of mice with COP-I in Freunds adjuvant in order to get a quick response for antibody production. These mice will get subsequent boosts of COP-I with adjuvant at two and four weeks following initiation of treatment. Control mice will receive normal saline or adjuvant alone. SJL mice will be pre-treated with Cop-I for 10 days and then injected with the Daniel's strain of Theiler's virus (2×10^5 PFUs) intracerebrally on day 0. The animals will be monitored clinically for neurological deficits weekly. Animals will be sacrificed on day 45 after infection and processed for detailed morphometic analysis to determine the extent of demyelination as per our previous protocols (18). Animals which develop severe paralysis, neurological deficits or are moribund will be sacrificed immediately, and processed for pathology. In brief, the brains are removed and sectioned in three coronal sections and embedded in paraffin. Sections are stained with hematoxylin and eosin and scored on a 4 point scale to determine the extent of inflammation and demyelination in various parts of the brain.

The entire spinal cord is removed, sectioned coronally into 1 mm blocks. Every other block is embedded in JB4 plastic and 1-2 micron sections stained with erichrome-cresyl-violet stain. The remainder of the sections are left in buffer for frozen sections to detect viral antigen and viral RNA by immunoperoxidase technique or in situ hybridization. A detailed morphologic examination is done of the entire spinal cord. The extent of demyelination, meningeal inflammation, or grey matter disease is expressed on a scale of 0 to 100 where 100 represents the presence of pathologic abnormalities in every quadrant of every spinal cord section examined. The primary endpoint for these experiments will be to determine whether Cop-I reduces the demyelination score. As secondary analysis we will determine the number of cell expressing of viral antigen or virus RNA per area of spinal cord by immunostaining and in situ hybridization. Serum will be collected at sacrifice from all animals and tested for antibody responses to Cop-I and against Theiler's virus antigens by ELISA. Delayed-type hypersensitivity responses to TMEV and Cop-I will be obtained by measuring increase in ear thickness to purified antigen. Ten mice will be included in each treatment or control group.

Experiment 2: **Does Cop-I treatment suppress the extent of demyelination once Theiler's virus-induced demyelination is established?** In the clinical situation, Cop-I treatment is initiated once demyelination is established and the diagnosis of multiple sclerosis is confirmed. Therefore, it is important to determine whether Cop-I reduces the extent of demyelination once Theiler's virus-induced disease is ongoing. For these experiments SJL mice will be infected with Theiler's virus as described previously. Fifteen days after infection (a time point in which demyelination is first evident in the spinal cord) animals will begin with treatments. The same experimental groups as described in Experiment 1 will be used. The animals will be monitored weekly for neurologic deficits and sacrificed on day 45 following infection. The primary and secondary endpoints will be as described in Experiment 1.

Experiment 3: **Does Cop-I promote CNS remyelination once severe demyelination an established?** One hypothesis of how Cop-I may be effective in MS is that it promotes a protective humoral immune response that enhances new myelin synthesis. This hypothesis will be tested directly in the Theiler's model system. SJL mice will be infected with Theiler's virus as described previously. Three to six months later (a time when demyelination is extensive in the spinal cord but there is minimal spontaneous remyelination) animals will receive Cop-I, normal saline, or mouse serum albumin. Animals will be treated for five weeks because experimental data in models of demyelination indicate that this is the time frame for CNS remyelination in rodents (19-21). At six weeks following treatment, animals will be sacrificed and the spinal cords processed for detailed morphometic analysis to determine the extent of demyelination and remyelination using 1 micron-thick Araldite-embedded sections as described previously in our protocols (17,22). The primary endpoint for these experiments will be determine the extent of remyelination in demyelinated areas. This is expressed as a percent: area of remyelination/area of lesion. Our previous experiments demonstrate that SJL mice infected with TMEV show 4 to 5% of the demyelinated area with significant remyelination (11). In our protocols using immunoglobulin therapy and monoclonal antibody therapy (15,17,23) , we have demonstrated that this number increases up to 30 to 35%. Ten mice will be included in each experimental group . Our previous experiments demonstrated we need 7 to 12 mice in each experimental group to show a statistically significant difference in remyelination between treatment and placebo.

Experiment 4:

Do antibodies directed against Cop-I promote CNS remyelination in the Theiler's model system? We have been intrigued with the possibility that patients who respond effectively to Cop-I develop high antibody titers to Cop-I. These immunoglobulins may be protective in disease and may ultimately promote

remyelination. We will test this hypothesis directly by passively transferring immunoglobulins directed against Cop-I to animals persistently infected with Theiler's virus. Animals will be infected with Theiler's virus for three to six months. At this time point when there is extensive demyelination but minimal spontaneous CNS remyelination, animals will receive purified immunoglobulin preparations reactive to Cop-I. The sources of immunoglobulin preparations are the following:

(a) High titer polyclonal rabbit immunoglobulins directed against Cop-I.

(b) Mouse monoclonal antibodies directed against Cop-I. A large panel of monoclonal antibodies directed against Cop-I which do or do not cross-react with myelin basic protein are available (24). The goal would be to pool these monoclonal antibodies into groups based on their specificities for treatment.

(c) Human polyclonal immunoglobulins directed against Cop-I. These are available from serum of MS patients treated with Cop-I. We propose to purify these immunoglobulins by affinity-chromotography to Cop-I. Those antibodies that stick to the column would be compared to immunoglobulins from the same patients that do not react to Cop-I. This would provide the ideal specificity control.

(d) As controls for these experiments we would use normal rabbit immunoglobulins which do not react with Cop-I. In addition we would need isotype-matched mouse monoclonal antibodies not reactive against Cop-I or myelin antigens.

A potential difficulty in these experiments is the possibility that mice will develop either an anti-rabbit or anti-human immunoglobulin immune response. Therefore treatment protocols using the rabbit or human immunoglobulins may have to be limited to one or two treatment injections. This could limit theoretically the potential efficacy of these immunoglobulins. However since the normal half-life of some immunoglobulins is frequently in the range of 8 to 10 days, treatment with these immunoglobulins once or twice during the five to six week period needed to promote CNS remyelination seems reasonable. In contrast, those experiments using the mouse monoclonal antibodies should

be less problematic. The protocol in this case would utilize intraperitoneal treatments two times a week, as per our previously established protocols using mouse monoclonal antibodies (15) and polyclonal immunoglobulins (17) directed against CNS antigens. The dosage of antibodies to be used in these experiments is empiric. Previous experiments using monoclonal antibodies against spinal cord antigens (94.03) have used 50 to 100 micrograms of monoclonal antibody injected IP twice weekly for five weeks for a total dose per mouse of 0.5 to 1.0 mg. For the experiments using affinity-purified antibodies directed against MBP, we used a dose of 30 μg of antibody given twice weekly for four weeks resulting in a total dose per mouse of 240 μg of purified immunoglobulin. The dose of Cop-I antibodies will need to be determined based on their affinity, avidity, reactivity, and purity. We predict utilizing 10 mice for each experimental group but it is likely that the experiments will need to be repeated two or three times depending on the results.

SIGNIFICANCE

These experiments have the potential of providing important new insights into the mechanism of how Cop-I may be effective in MS. Cop-I will soon be approved by the FDA for treatment of MS patients. If these experiments demonstrate that Cop-I induces CNS remyelination and more specifically that antibodies to Cop-I promote myelin repair, then this would provide a new avenue of investigation to develop therapeutic strategies to enhance natural humoral immunity to CNS and myelin antigens (14). At present, Cop-I has been shown to be effective in reducing exacerbations of multiple sclerosis. Our studies (25) examining biopsy tissues from patients with severe acute exacerbations demonstrate that remyelination is a significant component of the acute MS lesion. Therefore remissions may be associated with significant CNS remyelination. If Cop-I is shown to promote CNS remyelination, this may extend the rationale for the use of this compound in future clinical trials. For example, one could envision a clinical trial for

patients with a static non-evolving, non-progressive demyelinating neurological deficit

The goal would be to determine if Cop-I promotes functional recovery (i.e. by enhancing CNS remyelination). An alternative scenario is spinal cord injury in which there is frequently significant demyelination with preservation of axons resulting in a static non-evolving neurological deficit (26). In this situation, enhancement of remyelination may improve neurologic recovery. It is possible the Cop-I could be used in this framework, if found to promote CNS remyelination in the animal model.

VERTEBRATE ANIMALS

(1) Approximately 300 mice per year will be employed in these studies. SJL/J, (susceptible strains) will be obtained from the Jackson Laboratories, Bar Harbor, ME.

Estimate of Number of Mice Required		
Experiment #1 - Prevent Demyelination		N
Group 1	COP I SQ	10
Group 2	COP I + adjuvant	10
Group 3	Normal saline	10
Group 4	Adjuvant alone	10
Experiment #2 - Treat Demyelination Once Disease Established		
Group 1	COP I SQ	10
Group 2	COP I + adjuvant	10
Group 3	Normal saline	10
Group 4	Adjuvant alone	10
Experiment #3 - Promote Remyelination		
Group 1	COP I SQ	10
Group 2	COP I + adjuvant	10
Group 3	Normal saline	10
Group 4	Adjuvant alone	10
Experiment #4 - Ab Promotion or Remyelination		
Group 1	Polyclonal rabbit Ig anti Cop I	10
Group 2	Monoclonals against Cop I (Non-cross-reactive)	10
Group 3	Monoclonals against Cop I (cross reactive with MBP)	10
Group 4	Human polyclonal anti COP I	10

Group 5	Human polyclonal affinity purified anti COP I	10
Group 6	Human polyclonal Ig not reactive to COP I	10
Group 7	Normal rabbit serum	10
Group 8	Isotype control monoclonal antibodies	10
Group 9	Normal human IG control	10

TOTAL - minimun 220 mice
It is very likely that experiments may need to be repeated = total 440 mice/over 2 years. Therefore we will plan to utilize 300 mice/year to allow for death following infection and the possibility that some mice may not develop sufficient demyelination to access remyelination (This is usually < 5% of mice).

(2) Mice will be infected at 5 weeks of age intracerebrally with 2×10^5 PFU of TMEV. Mice will be sacrificed at various time points as described in detail in the experimental design section of this grant. If mice develop severe neurologic deficits or become moribund, they will be sacrificed immediately.

(3) TMEV-infected animals are housed in a special animal facility on the 5th floor of the Medical Science Building designed for infected animals. This facility is maintained by full-time professionals and veterinary staff. The principal investigator will periodically check the condition of the animals. The facility has been accredited by AAALAC. Veterinary care includes daily observation and surveillance for assessment of animal health, appropriate methods of disease control, recommendation as to appropriate handling, restraint, anesthesia and euthanasia.

(4) We will make all efforts to limit distress, pain, and injury to these mice except for what is unavoidable in the conduct of our research. Virus injection (intracerebral) will be performed while animals are lightly anesthetized with metafane (methoxyfluorane) in a biohazard hood. Virus injection involves using a 27 gauge needle with a Hamilton syringe which delivers a 10 µl of purified virus. This results in approximately 95% incidence of demyelination with only a rare fatality following injection. Animals will be monitored daily during the course of disease. All paralyzed

animals will receive special food and water from easily accessible containers. Generally the animals remain alert and responsive during this period.

(5) Animals will be killed by overdosage with intraperitoneal pentobarbital. The method is consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association. Certain mice selected for electron microscopic studies will be anesthetized with pentobarbital and perfused with fixative. The chest is opened and the fixatives are perfused through the heart. The animal dies painlessly without recovery from anesthesia. The brain and spinal cord are then removed and prepared for histology.

METHODS

1. Virus. The Daniel's DA strain of TMEV was obtained from Drs. J. Lehrich and B. Arnason (University of Chicago) after eight passages in BHK cells. The virus was passaged as additional four times at a multiplicity of infection of 0.1 plaque-forming units (PFU) per cell . Cell-associated virus was released by freeze-thawing the cultures followed by sonication. The lysate was clarified by centrifugation and stored in aliquots at -70°C. This virus isolate causes white matter pathology (demyelination) without destruction of anterior horn cells.

2. Demyelination scores on JB-4-embedded sections. Animals are injected intracerebrally with 2×10^5 PFU of the Daniel's strain of Theiler's virus in 10 µl of volume. On specified day following infection, mice are perfused by intracardiac puncture with Trump's fixative (phosphate-buffered 4% formaldehyde with 1.5% glutaraldehyde, pH 7.2). The entire spinal cord is removed and sectioned precisely into 1 mm blocks in cross-sections. Each spinal cord block is embedded in glycol-methacrylate (JB-4) and processed to provide 2 µm thick glycolmethacrylate-embedded sections. Spinal cord sections are stained with a modified erichrome stain with cresyl violet counterstain to detect demyelination in plastic-embedded sections. A detailed morphologic analysis is

performed on each of 10-15 coronal spinal cord sections from each animal. A pathologic score based on neuronal inflammation, meningeal inflammation, or demyelination in the spinal cord white matter is obtained for each animal. Pathologic score for each animal are expressed as the percentage of quadrants showing pathology divided by the total number of quadrants examined. The maximum pathologic score, 100, indicates the presence of pathologic abnormalities in each quadrant of every spinal cord section examined from each mouse. All analysis is done on coded sections in a non-biased fashion. Differences between groups is analyzed using Student's t test.

3. Treatment protocol to promote remyelination. Demyelination is induced in female SJL/J mice, ages four to six weeks, from the Jackson Laboratory, Bar Harbor, ME. Mice are inoculated intracerebrally with 2×10^5 plaque-forming units of DA virus in a volume of 10 µl. Mice infected chronically with TMEV (3-6 months) are assigned randomly to groups of treatment. Treatment with purified Igs or mAbs is given by intraperitoneal (IP) route two times per week for five weeks or once by the intravenous route (IV). The animals are sacrificed on the sixth week, and processed for electron microscopy.

4. Preparation of tissue for light and electron microscopy. Mice receiving Igs are anesthetized with 0.2 mg of pentobarbital, exanguinated by intracardiac puncture, and killed by intracardiac perfusion with Trump's fixative (phosphate-buffered 4% formaldehyde containing 1.5% glutaraldehyde, pH 7.2). Spinal cords are dissected carefully from the bony canal, obex to conus medullaris, and the entire cord is sectioned precisely into coronal blocks 1 mm thick. Every third block is postfixed in 1% osmium tetroxide for two hours and embedded in Araldite (Polysciences, Warrington, PA). The remainder of the sections are left unosmicated for future immunolabeling studies. Cross-sections (1 µm) from each block are stained with 4% p-phenylenediamine. Selected areas are trimmed and prepared for electron microscopy.

5. Morphometry of demyelination and remyelination spinal cord. A detailed morphometric analysis is performed on 1 μm spinal cord cross-sections with a Zeiss interactive digital analysis system (ZIDAS) and camera lucida attached to a Zeiss Photomicroscope. This equipment is in the principal investigator's office and is used on a daily basis for analysis of most morphologic material. The following data is quantitated from each section: a) area of white matter, b) number and area of demyelinating lesions, c) number and area of lesions undergoing remyelination by oligodendrocytes, d) number and area of lesions undergoing remyelination by peripheral nervous system Schwann cells, e) percentage of total demyelinated area showing remyelination by oligodendrocytes. Data is analyzed using student's t test against control. Abnormally thin myelin sheaths in reference to axon diameter is used as the criteria for CNS remyelination by oligodendrocytes.

6. Mayo Clinic Optical Morphology Laboratory. The Mayo Clinic optical morphology lab includes a Zeiss IBAS 2000 image analysis system with a host computer, image processor, hard disk and floppy disk drives, keyboard, data monitor, digitizer tablet, cursor, color monitor and printer. Digitized images can be produced from any of the following currently available derives: (1) Zeiss Axiophote upright microscope with transmitted light for brightfield, Nomarski DIC, and phage contrast; and with epifluorescence for use with various fluorochromes. The Axiophote is equipped with a photomultiplier based 35 mm camera module. (2) Zeiss IM35 inverted microscope with transmitted light for brightfield, Nomarski DIC, and phase contrast; and epifluorescence for visible or U.V. wavelengths with a special quartz raypath. (3) Light box and table designed to allow imaging of photographs, negatives, etc., through the use of a Zeiss 100 mm macro lens. Input from these devices to the IBAS 2000 is achieved through the use of video cameras. Currently available are the Hamamatsu newvicon, silicon intensified target (SIT) and photon counting camera. Images are digitized to 256 different gray levels and displayed on the monitor. Gray levels can also be represented as pseudo

colors. The images can be transformed by user defined parameters in such a way as to retain, eliminate, or enhance certain structures. Desired parameters are measured such as area, shape, number, etc. Data is displayed on the monitor, printed or stored on disk for further evaluation. Additional programs are available for statistics and classification of image specific data. Digitized images themselves may be stored on cartridges for further processing and evaluation. Hard copy of images are produced with a Matrix Instruments Model 3000 color graphic recorder equipped with a 35 mm camera or polaroid film back for instant 4 x 5 pictures.

7. Immunoperoxidase straining for TMEV antigen in spinal cord sections. Spinal cord blocks (1-2 mm thick) from animals previously perfused with Trump's fixative and stored in 0.1M phosphate buffer. Blocks are rinsed in 0.1M Tris buffer with 25mM hydroxylamine, pH 7.4, treated with 10% DMSO in the same buffer for 1 hr and quick-frozen in isopentane chilled in liquid nitrogen. Ten micron cryostat sections are cut and transferred to gelatinized glass slides. Frozen sections are reduced with 1% sodium borohydride in 0.1M Tris buffer with 25mM hydroxylamine, pH 7.4 at 4°C and re-fixed with 95% alcohol/5% glacial acetic acid. Sections are immunostained with polyclonal antiserum to purified DAV virions by avidin-biotin immunoperoxidase technique (Vector Laboratories, Burlingame, CA). Sections are counter-stained with hematoxylin to detect inflammatory infiltrates and areas of pathology. Brain sections processed in the same way from a neonatal mouse infected with TMEV serve as a positive control.

8. In situ hybridization from virus RNA. In situ hybridization is performed on frozen and paraffin embedded sections on Denhardt treated acetylated slides. Frozen sections are prepared from spinal cord of persistently infected mice. Sections are cut at 6 μ thick onto precoated Poly-d-lysine slides and are fixed in PLP (.01M percodate - 76 mM lysine - 0.5% paraformaldehyde). Paraffin sections are placed onto Poly-d-lysine precoated slides and allowed to dry overnight at 37°C before hybridizing. Prior to immunocytochemistry and in situ hybridization, slides from paraffin embedded tissue are

immersed twice for 5 min in xylene, and twice each for 5 min in graded series of alcohol to remove paraffin.

Tissue sections are permeated by 4 min in PBS with 0.1% Triton-X 100, washed 2 times in PBS, and immunostained with appropriate primary antibody by avidin-biotin immunoperoxidase technique as described. After immunocytochemistry, slides are washed twice in distilled water and treated as follows: 20 min at 22°C in 0.2N HCL; 30 min at 70°C in 300 mM NaCl, 30 mM Na Citrate, pH 7.0 (2 x SSC); 15 min at 37°C in 20 mM Tris-HCL, pH 7.4 with 2 mM $CaCl_2$ containing 1 ug of proteinase K per ml; 10 min at 22°C in 0.1M triethanolamine (pH 7.5) containing 0.25% acetic anhydride. Slides are washed briefly in distilled water between treatments. After the acetylation step, slides are dehydrated in 70% and 95% ethanol, respectively.

Slides are hybridized with 2 ng/ul of a ^{35}S-labeled 253-bp (nucleotides 3053 to 3305) and 363-bp (nucleotides 3306 to 3668) cDNA probes corresponding to VP1 of TMEV (DA strain). The cDNA probes were obtained by double digesting the VP1 plasmid with *Kpn*I and *Sal*I restriction enzymes and radiolabeling the probes with between 0.5×10^8 to 0.8×10^8 cpm of [___-^{35}S]dATP per µg of DNA by nick translation. Hybridization is carried out overnight at 37°C in hybridization mixture containing 50% formamide, 10 mM Tris-HCL (pH 7.4), 1 mM EDTA, 5% polyethylene glycol, 100 um aurin tricarboxylic acid, 0.6 mNaCl, 0.2M HEPES (pH 7.2), 1 x Denhardt's medium, 10 ug/ul yeast tRNA, 1 mg/ml poly (A), and 10 mM dithiothreitol. At the end of the hybridization period, slides are washed for 5 min in 50% formamide, 10 mM HEPES (pH 7.2), 1 mM EDTA and 0.6 M NaCl (HWM); 5 min in 2 x SSC at 22°C; 1 hr in SSC at 70°C and 1-3 days in HWM at 22°C. Washed slides are dehydrated in graded alcohols containing 300 mM ammonium acetate, air-dried and immersed in NTB-2 emulsion (Eastman Kodak Co., Rochester, NY). After 3-5 days of exposure - at 4°C, slides are developed with Kodak D-19 and fixed with Kodak fixer. Slides are counterstained with hematoxylin

9. Brain pathology scores. Brains from perfused animals are cut into three coronal sections, and embedded in paraffin. Sections are stained with hematoxylin/eosin. The cerebellum, brain stem, hippocampus, striatum, cerebral cortex, corpus callosum and meninges were graded independently on a four-point scale for the presence of inflammation, demyelination and necrosis (0, no pathologic abnormalities; 1, minimal inflammation with <25 cells per 40x high-power field (HPF); 2, moderate inflammation with 25 to 50 inflammatory cells per HPF without parenchymal injury; 3, intense inflammation with >50 inflammatory cells per HPF with definite demyelination or early necrosis; and 4, intense inflammation and widespread necrosis.

10. Delayed-type hypersensitivity (DTH) responses to Theiler's virus. TMEV-specific DTH responses are elicited in the ear by intradermal injection of 10 µl of uv-inactivated virus (titer prior to inactivation of 2×10^8 PFU/ml). Forty-eight hours after challenge the increase in ear thickness over the prechallenge measurements in each animal are determined with a Peakcock Dial Gauge G-50 micrometer (Ozaki Manufacturing Co.) and expressed in units of 10^{-2} mm. As control, a lysate of uninfected L2 cells which were used to propagate the virus is inoculated into the ears of mice.

11. Antibody titers against TMEV. Anti-TMEV antibodies in the serum are measured by an indirect enzyme-linked immunosorbent assay (ELISA) that uses purified TMEV (DA strain) virus as the antigen.

REFERENCES

1. **Johnson, K. P., B. R. Brooks, J. A. Cohen, C. C. Ford, J. Goldstein, R. P. Lisak, L. W. Myers, H. S. Panitch, J. W. Rose, R. B. Schiffer, and et al.** 1995. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group [see comments]. *Neurology 45*:1268.
2. **Teitelbaum, D., A. Meshorer, T. Hirshfeld, R. Arnon, and M. Sela.** 1971. Supression of experimental allergic encephalomyelitis by a synthetic polypeptide. *Eur J Immunol 1*:242.
3. **Arnon, R., D. Teitelbaum, and M. Sela.** 1989. Suppression of experimental allergic encephalomyelitis by Cop1-relevance to multiple sclerosis. *Isr J Med Sci 25*:686.
4. **Racke, M. K., R. Martin, H. McFarland, and R. B. Fritz.** 1992. Copolymer-1-induced inhibition of antigen-specific T cell activation: interference with antigen presentation. *J Neuroimmunol 37*:75.
5. **Aharoni, R., D. Teitelbaum, and R. Arnon.** 1993. T suppressor hybridomas and interleukin-2-dependent lines induced by copolymer 1 or by spinal cord homogenate down-regulate experimental allergic encephalomyelitis. *Eur J Immunol 23*:17.
6. **Teitelbaum, D., R. Milo, R. Arnon, and M. Sela.** 1992. Synthetic copolymer 1 inhibits human T-cell lines specific for myelin basic protein. *Proc Natl Acad Sci USA 89*:137.

7 **Fridkis-Hareli, M., D. Teitelbaum, E. Gurevich, I. Pecht, C. Brautbar, O. J. Kwon, T. Brenner, R. Arnon, and M. Sela.** 1994. Direct binding of myelin basic protein and synthetic copolymer 1 to class II major histocompatibility complex molecules on living antigen-presenting cells--specificity and promiscuity. *Proc Natl Acad Sci U S A 91*:4872.

8. **Miller, D. J. and M. Rodriguez.** 1995. Spontaneous and induced remyelination in multiple sclerosis and the Theiler's virus model of central nervous system demyelination. [Review]. *Microsc Res Tech 32*:230.

9. **Rodriguez, M., E. Oleszak, and J. Leibowitz.** 1987. Theiler's murine encephalomyelitis: a model of demyelination and persistence of virus. *Crit. Rev. Immunol. 7*:325.

10. **Rodriguez, M., V. A. Lennon, E. N. Benveniste, and J. E. Merrill.** 1987. Remyelination by oligodendrocytes stimulated by antiserum to spinal cord. *J. Neuropathol. Exp. Neurol. 46*:84.

11. **Rodriguez, M. and V. A. Lennon.** 1990. Immunoglobulins promote remyelination in the central nervous system. *Ann. Neurol. 27*:12.

12. **Rodriguez, M.** 1991. Immunoglobulins stimulate central nervous system remyelination: electron microscopic and morphometric analysis of proliferating cells. *Lab Invest 64*:358.

13. **Rodriguez, M.** 1992. Central nervous system demyelination and remyelination in multiple sclerosis and viral models of disease. *J. Neuroimmunol. 40*:255.

14. **Miller, D. J. and M. Rodriguez.** 1995. A monoclonal autoantibody that promotes central nervous system remyelination in a model of multiple sclerosis is a natural autoantibody encoded by germline immunoglobulin genes. *J Immunol 154*:2460

15. **Miller, D. J., K. S. Sanborn, J. A. Katzmann, and M. Rodriguez.** 1994. Monoclonal autoantibodies promote central nervous system repair in an animal model of multiple sclerosis. *J Neurosci 14*:6230.

16. **Miller, D. J., K. Asakura, and M. Rodriguez.** 1995. Experimental strategies to promote central nervous system remyelination in multiple sclerosis: insights gained from the Theiler's virus model system. [Review]. *J Neurosci Res 41*:291.

17. **Rodriguez, M., D. J. Miller, and V. A. Lennon.** 1996. Immunoglobulins reactive with myelin basic protein promote CNS remyelination. *Neurology 46*:538.

18. **Rodriguez, M. and C. D. David.** 1995. H-2Dd transgene supresses Theiler's virus-induced demyelination in suscectible strains of mice. *J. Neurovirol 1*:111.

19. **Crang, A. J. and W. F. Blakemore.** 1991. Remyelination of demyelinated rat axons by transplanted mouse oligodendrocytes. *GLIA. 4*:305.

20. **Ludwin, S. K.** 1989. Evolving concepts and issues in remyelination. *Dev. Neurosci. 11*:140.

21. **Ludwin, S. K.** 1988. Remyelination in the central nervous system and the peripheral nervous system. *Adv. Neurol. 47*:215.

22. **Miller, D. J., C. Rivera-Quinones, M. K. Njenga, J. Leibowitz, and M. Rodriguez.** 1995. Spontaneous CNS remyelination in beta(2) microglobulin-deficient mice following virus-induced demyelination. *J Neurosci 1545*:8345.

23. **van Engelen, B. G., D. J. Miller, K. D. Pavelko, O. R. Hommes, and M. Rodriguez.** 1994. Promotion of remyelination by polyclonal immunoglobulin in Theiler's virus-induced demyelination and in multiple sclerosis. [Review]. *J Neurol Neurosurg Psychiatry 57 Suppl*:65.

24. **Teitelbaum, D., R. Aharoni, M. Sela, and R. Arnon.** 1991. Cross-reactions and specificities of monoclonal antibodies against myelin basic protein and against the synthetic copolymer 1. *Proc Natl Acad Sci U S A 88*:9528.

25. **Rodriguez, M. and B. Scheithauer.** 1994. Ultrastructure of multiple sclerosis. *Ultrastruct Pathol 18*:3.

26. **Blight, A. R.** 1993. Remyelination, revascularization, and recovery of function in experimental spinal cord injury. [Review]. *Advances in Neurology 59*:91.

rincipal Investigator : RODRIGUEZ,M.
unding Sourc : TEVA Pharmaceutical
itle : Copolymer I (Cop-I) and the Promotion of Central Nervous System Remyelination

Grant Contract Id : -1

		% EFFORT	BASE SALARY	10/1/96 9/30/97 AMOUNT	10/1/97 9/30/98 AMOUNT	TO AMO
ERSONNEL						
IERCE,M.L	Histology Technician	50%				
ODRIGUEZ,M. MD	Principal Investigator	5%				
ECKLEIN,L J	Research Technician	50%				
o Be Named ellow	Pre-Doctoral Research	100%				
	Total PERSONNEL			$71,750	$74,620	$14
	Benefits (19.60% of salaries)			$14,063	$14,626	$2
	Total Personnel (including benefits)			$85,813	$89,246	$17
UPPLIES						
urchase of Mice ($9.75 x 300/year)				2,625	2,730	
orphology (osmium, plastic embedding)				3,000	3,120	
eneral Laboratory Supplies				1,000	1,040	
	Total SUPPLIES			$6,625	$6,890	$1
)TI EXPENSES						
aintenance of Mice (200 mice x $16.98/mouse/year)				5,698	5,926	1
ublication Costs				500	520	
	Total OTHER EXPENSES			$6,198	$6,446	$1
OTAL DIRECT COST				$98,636	$102,582	$20
NDIRECT COST - 43.10% of MTDC				$42,512	$44,213	$8
OTAL COST				$141,148	$146,795	$28

udget Id 3977 Grant_Id 0

.dministrator Name : MC CLURE,O.E
oot Note
rinted On : Sep 19, 1996

Appendix B

1. Payments shall be made by Teva directly to the Institute upon receipt of an invoice in accordance with law and in accordance with terms of payment set forth in paragraph 4 below.

2. The total budget for the Project is US$ 287,943 (US$ 141,148 for the first year and US$ 146,795 for the second year).

3. Payments will be paid in four equal quarterly payments. The last payment every year will be made after submission of a scientific report to Teva.

4. Payments will be effected 30 days after the end of the month in which the invoice was submitted and once payments are authorized.